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Letters to the Editors

Comments on: Effect of Turgor Pressure on Water Permeability of *Allium cepa* Epidermis Cell Membranes

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We should like to comment on the paper by Palta and Stadelmann [3] concernin the measurement of the hydraulic conductivity, L_p, in onion epidermal cells. The author determined L_p by eluting monocellular epidermal strips soaked with tritiated water an did not find an effect of the osmotic pressure of the medium on L_p. On the one hand Palta and Stadelmann feel that this result contradicts the findings of Zimmermann an Steudle [5] who reported a remarkable increase of L_p for different *Characean* species an also for the marine alga *Valonia utricularis* in the range of low cell turgor pressure P, on approaching the plasmolytic point. On the other hand, they feel their work i confirmed by the results of the same authors, who found no pressure dependence o the hydraulic conductivity in the bladder cells of *Mesembryanthemum crystallinum* ove the whole range of P (0 to 5 bar) [4]. Possibly Palta and Stadelmann are not fully awar of the interpretation of these different effects of turgor pressure [6], which are by n means contradictory. For the discussion of the reliability of L_p values of higher plan cells obtained by different methods which may vary by 4 orders of magnitude, it is extremel important to know whether L_p is pressure-dependent.

Palta and Stadelmann stress the argument that in the experiments on *Characean* inte nodes and *V. utricularis* the cell membrane was damaged at low P (0 to 2 bar) due t the experimental procedure and that the increase of L_p is an artefact. This is certainl not true. Zimmermann and Steudle used their pressure probe technique [4, 5] to determin P and L_p directly from pressure relaxation experiments which could be induced eithe by changing the osmotic or hydrostatic pressure gradient. In these measurements (1) th cells behaved like ideal osmometers, (2) *Characean* cells showed cytoplasmic streamin over the entire pressure range, and (3) the membrane potential which was measured simu taneously in *V. utricularis* cells was not affected by the experimental procedure. This exper mental evidence demonstrates that the algal cells were intact at low P and did not becom leaky. Furthermore, it was shown [5] that both the concentration of the medium *an* the turgor pressure affect L_p of *Characean* cells in a different way. In osmotic experimen the increase in the external concentration caused a reduction of L_p which had also bee found to be the case in transcellular osmosis measurements [1]. However, at low P th concentration effect could be compensated or even overcompensated by the inverse effe of turgor. This is shown in Fig. 1, where the pressure dependence of L_p of a *Nitel flexilis* internode is shown both in the presence and absence of an osmoticum (0.1 sucrose) in the external solution. Due to the concentration dependence of L_p the grap of $L_p=f(P)$ obtained in artificial pond water (APW) is shifted to lower turgor pressure but does not change its hyperbola-like shape. This result strongly supports pressure-depe

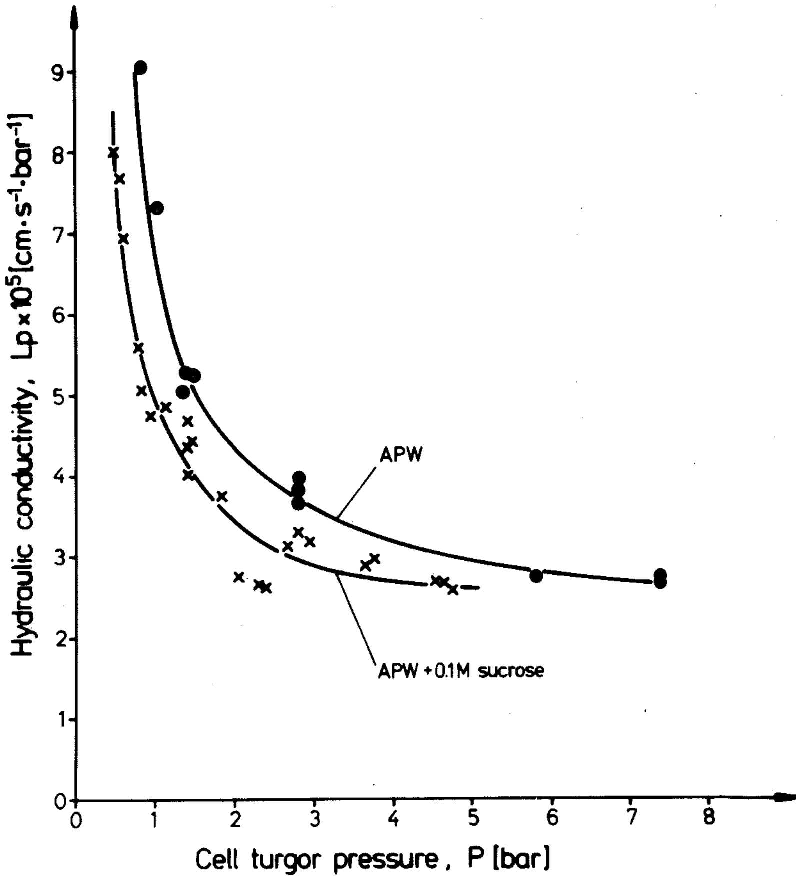

Fig. 1. Effect of cell turgor pressure, P, on the hydraulic conductivity, L_p, of a single internodal cell of *Nitella flexilis* in artificial pond water (APW) (composition: 1 mM NaCl; 0.1 mM KCl, $CaCl_2$, and $MgCl_2$, respectively) and in APW+0.1 M sucrose. The hydraulic conductivity was calculated from exosmotic water flows induced by hydrostatic pressure gradients with the aid of the pressure probe technique [4, 5]

dent changes of the hydraulic conductivity, rather than a mechanical rupture of the cell membrane, which would occur at the same P, independently of the nature and concentration of the solute in the medium.

The effect of turgor pressure on L_p has been discussed in terms of structural changes of the cell membranes [5] and in terms of an interaction between ion fluxes and water flow [6]. In *V. utricularis* the potassium uptake increases sharply in the low pressure range [*cf.* 6] and thus exhibits a pressure dependence very similar to that of L_p in this range. Theoretically, it can be shown for the steady state that in the presence of a net ion (solute) flux, J_s, an additional water (volume) flow, J_w, will be generated which may be substantial, if J_s is high and the volumetric elastic modulus, ε, of the cell is low. If the rate of exchange of the solutes across the cell membrane is much slower than

that of water, the relation between J_s and J_w will be given by [6]:

$$J_s = \frac{\varepsilon + \Pi^i}{RT} J_w \qquad (1$$

where Π^i is the osmotic pressure of the cell sap.

Eq. (1) was derived for an impermeable solute in the presence of an active stationar solute flow and a stationary water flow. Using Eq. (1) it is possible to evaluate the apparer resistance of the cell membrane to water, dP/dJ_w, under these conditions ([6], see als [2]):

$$\frac{dP}{dJ_w} = \frac{1}{L_p} - \frac{J_s \cdot RT}{J_w^2}. \qquad (2$$

Eq. (2) states that dP/dJ_w depends on both L_p and on the direction and magnitude c J_s. At least qualitatively, Eq. (2) can explain the increase of the water conductivity i the low pressure range observed in *Valonia* under nonstationary conditions. J_s is inwardl directed (i.e., it is negative) and strongly pressure-dependent. Thus, the hydraulic conductiv ity calculated from dP/dJ_w will be higher, if L_p is calculated assuming that $J_s = 0$. Fc *Characean spp.* a similar consideration may be valid. It should be pointed out that th apparent L_p value calculated for $J_s = 0$ is the physiologically relevant parameter for th water relations of plant cells instead of the thermodynamic value of L_p.

If the hydraulic conductivity is independent of turgor pressure, this only indicate that there is either no coupling between water flow and active solute flow in these cel or that the coupling coefficient and the magnitude of solute flow are unaffected by over the entire pressure range. In general, interactions between active solute (ion) transpo and water transport have to be taken into account when the L_p of plant cells is determine in the presence of active solute flows. Some of the discrepancies as regards the absolu value of L_p in the literature may be due to the neglect of this important point. Furthermor turgor-dependent structural changes of the plasmamembrane such as folding or compressic may also result in changes of L_p. Therefore, if the effect of turgor pressure on the water pe meability is to be studied, it is undoubtedly necessary to determine P directly and to separa the pressure effect from concentration effects (*see* Fig. 1).

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Reply to *Comments on*: Effect of Turgor Pressure on Water Permeability of *Allium cepa* Epidermis Cell Membranes

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The comments on our paper (Palta and Stadelmann, 1977) by Steudle and Zimmermann o not provide any convincing arguments to support their earlier conclusions (Steudle nd Zimmermann, 1974). Following are the reasons for this assertion:

I. The variability of L_p values with cell species and/or physiological conditions should ot be confused with the variability of L_p caused by errors in the various experimental ıethods used on the same cell material under the same physiological conditions. The laimed variability of L_p values for higher plant cells by 4 orders of magnitudes cannot e corroborated in the literature, where reliable values indicate a 15- to 20-fold maximum ariation of L_p between different types of cells (Stadelmann, 1963, p. 698*f*). Variations ı L_p values for different cell material must be expected because of the differences in ıe composition and structure of the membranes of different cell species.

II. Cytoplasmic streaming, an established criteria for cell viability, is frequently observed ı injured cells, where it may be even more intense. Likewise perfect semipermeability f the protoplasm layer for the vacuolar solutes does not exclude changes in water permeabil-:y and other transport parameters. Steudle and Zimmermann are probably not aware ıat membrane alterations can occur without affecting cytoplasmic streaming and semiper-ıeability. It was recently shown by Palta *et al.* (1977*a*, 1977*b*) that freeze injured cells ehave like ideal osmometers and exhibit cytoplasmic streaming just like uninjured cells, /hereas the active transport systems were damaged.

The factors causing the membrane damage in the punctured *Nitella* cell cannot yet e assessed, partly because of lack of details about the puncturing procedure. The sharp ıcrease in L_p measured at zero turgor in these cells could be caused by membrane rupture roduced by the sudden pressure increase (1–2 atm pressure applied by a pressure probe sed by Steudle and Zimmermann) required for the L_p measurement. Such rupture may ccur instantaneously and thereby result in a very short relaxation time (very high value f L_p). A temporary membrane rupture may repair very quickly and will not affect semiper-ıeability and protoplasmic streaming. Osmotically induced breaking of red blood cells or preparing ghosts is a very good example of membrane rupture under pressure followed y a quick repair. Another possible reason for the increase in L_p would be a lower structural tability of the cell membrane and/or the vacuolar membrane because of the drastic change

of the ionic content of the cell sap vacuole by the lowering of the cell sap concentrati
using cell puncture procedure.

III. It will be quite a coincidence that in the experiments conducted by Dainty a
Ginzburg (1964), Tazawa and Kamiya (1966), and Palta and Stadelmann (1977), the conce
tration of the external solution always accurately compensated the corresponding increa
in L_p. The L_p is expected to increase sharply at the lower turgor pressure according
Steudle and Zimmermann which was not found by any of these authors. Different metho
and different plant materials were used by these authors to measure L_p. Furthermo
water permeability constant values for *Allium cepa* inner epidermis cells found at plasmoly
ing concentrations (Url, 1971) are very close to the diffusional water permeability valu
found by Palta and Stadelmann (1977).

IV. There is no evidence for membrane folds at low turgor pressure. Electron microscop
pictures of plasmolyzed cells showed absence of any fold (Sitte, 1963). Furthermore,
folding is the explanation, it should have the same effect in bladder cells of *Mesembryanth
mum crystallinum*. No effect on L_p was found at low turgor in these cells (Steudle *et a*
1975). Active transport has been found to be sensitive to turgor pressure changes, a
this experimental evidence can be used to explain the well-known phenomenon of osmoreg
lation in algal cells (Bisson & Gutknecht, 1975). This however, does not necessarily mean a
effect of turgor on L_p. Turgor changes induce active transport of ions which can genera
some water flux in the direction of the ion flux. This water flux coupled to the io
flux does not indicate that the hydraulic conductivity of the membrane (L_p) has change
also.

V. The graph in the letter of Steudle and Zimmermann is no unequivocal evidenc
for a pressure dependence of L_p. The lower L_p values for the same turgor pressure i
0.1 M sucrose+APW supports rather the suggestion of Palta and Stadelmann (1977) th
the increase in L_p with lowering P is caused by membrane alteration. Sucrose is know
for its protective action on cell membranes; thus, L_p will be lower at the same pressu
P in presence of sucrose in the medium. Tests with solutions of substances which do no
have such membrane effect, or more generally, experiments using a different method fo
measuring L_p would be required to demonstrate unequivocally any pressure dependenc
of L_p in *Nitella* cells.

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For all other references, see:

Palta, J.P., Stadelmann, E.J. 1977. Effect of turgor pressure on water permeability of *Allium cep* epidermis cell membranes. *J. Membrane Biol.* **33**:231